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Influence of Abiotic Factors on the Phytochemical Profile of Two Species of *Artemisia*: *A. herba alba* Asso and *A. mesatlantica* Maire

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Abstract: The species of *Artemisia* are well known in the Mediterranean region, especially in Morocco, for their traditional uses and health benefit. In this study, we were interested in two species of *Artemisia*, namely *A. herba alba* Asso and *A. mesatlantica* Maire. These species were collected from different soils of the Central Middle Atlas (loamy, stony, limestone and rocky soil) with different atmospheres. Extraction of essential oils from the leaves and flowering tops was carried out by hydrodistillation in Clevenger apparatus. Chemical composition analysis was further carried out using gas chromatography coupled with mass spectrometry (GC-MS). Principal component analysis (PCA) was performed to determine the similarities and dissimilarities in the chemical compositions of these six essential oils. The results obtained showed that the essential oil contents extracted from the flowering tops vary from one species to another according to the place of harvest, altitude, soil type and climate. The essential oil yield is between 0.84% and 2.19% (mL/100 g). Chemical analysis revealed that the chemotype of *A. herba alba* in limestone soil with a subhumid to humid atmosphere is *trans*-thujone (33.78%), while camphor (46.19%) is for limestone soil with a semi-arid atmosphere, vetivenic acid (14.91%) and davana ether (14.64%) are for limestone soil with a semi-arid and arid atmosphere and camphor (18.39%) is for loamy and stony soil with a semi-arid atmosphere. As for *A. mesatlantica* from a rocky soil on limestone with a subhumid to humid atmosphere, the main component is camphor (44.86%), and that of limestone soil with a subhumid to the humid atmosphere *trans*-thujone (41.08%). In addition, HCA affirmed the PCA and allowed us to distinguish between four groups. Our findings observed differences in the chemical compositions of the isolated essential oils most likely related to many factors such as the climates in the regions of the samples collected, altitudes and soil types.

Keywords: chemotypes; essential oil; hydrodistillation; GC/MS; chemical composition; PCA; HCA; plant diversity; abiotic factors

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1. Introduction

Morocco has several *Artemesia* called "Chih" in Arabic and "Izri" or "ifssi" in Tamazighte. *Artemisia* are part of the Asteraceae family, which contains between 200 and 400 herbaceous

species in arid, semi-arid, sub-humid and humid zones [1]. There are 19 species in Morocco [2], the most important of which are *A. alba chitachensis, A. atlantica var. maroccana, A. flahauti, A. mesatlantica, A. negrei, A ifranensis, A. herba alba, A. arborescens* and *A. absinthium*. Studies of the chemical composition of essential oils (EOs) and chemotypes have revealed a high degree of polymorphism [3]. In Morocco, 16 chemotypes have been identified, with monoterpenes as the major constituents [4]. We also note the presence of sesquiterpene alcohols, sesquiterpene acids, flavonoids, coumarins, polyenes, sterols and triterpenes [5]. The major components of *Artemisia* EOs are *trans*-thujone, *cis*-thujone, camphor, 1, 8-cineole, α-terpineol, chrysanthenone, camphene, α-pinene and davanone. Variations in chemical composition are the result of many factors such as the geographic region, season and environmental and climatic conditions [1], along with genetic factors [6]. Most actors (herbalists, cooperatives, associations, etc.) market local products (plants, hydrosols, etc.) with numbered labels, without any scientific denomination of the exact species, nor of the region of harvest. It should be noted that the chemical composition differs between species or even very similar subspecies of the same genus. It is, therefore, imperative to ensure the botanical identity of the species and determine its chemical composition to avoid any risk of toxicity. Morocco supplies 90% of the sagebrush essential oils to the world market, used in the cosmetics and perfume industries [7]. To enhance the essential oil of sagebrush, two spontaneous species collected at five sites in the Fez-Meknes-Morocco region were chosen. The purpose of this study was to participate in the socioeconomic development of this region's population, to enable them to promote and market their local products. Our contribution is to provide answers as to the scientific names of the sagebrush species, the yields of the essential oils and their qualitative and quantitative chemical compositions.

2. Materials and Methods

2.1. Study Zone

The central Middle Atlas of Morocco has a very varied bioclimate (climate, soil, relief, etc.), with forest cover and remarkable flora. This constitutes a protective and productive heritage, as well as a genetic reservoir of biodiversity. This regional flora, thus rich in aromatic and medicinal plants, can be developed as a source of products with high added value for the rural populations of the regions of Boulemane and Ifrane. The territory of our study comprised five sites: Serghina, Oulad aliyoussef, Enjil, Guigou and Timahdit. These sites are grouped in the central Middle Atlas mountain zone (Figure 1).

2.2. Plant Material

The studied samples were collected at the time of their flowering (months of March and April 2017 and 2018) (Table 1). Then, they were dried in the shade for ten days. The botanical identification of species was carried out at the Floristics Laboratory of the Scientific Institute in Rabat by Hamid Khamar. Voucher specimens were deposited in the National Herbarium (RAB) of the Scientific Institute RABAT, with the code number of *A. herba alba* as RAB113451 and that of *A. mesatlantica* as RAB113452. Morphological aspects of *A. herba alba* Asso (A) and *A. mesatlantica* Maire (B) are presented below (Figure 2).

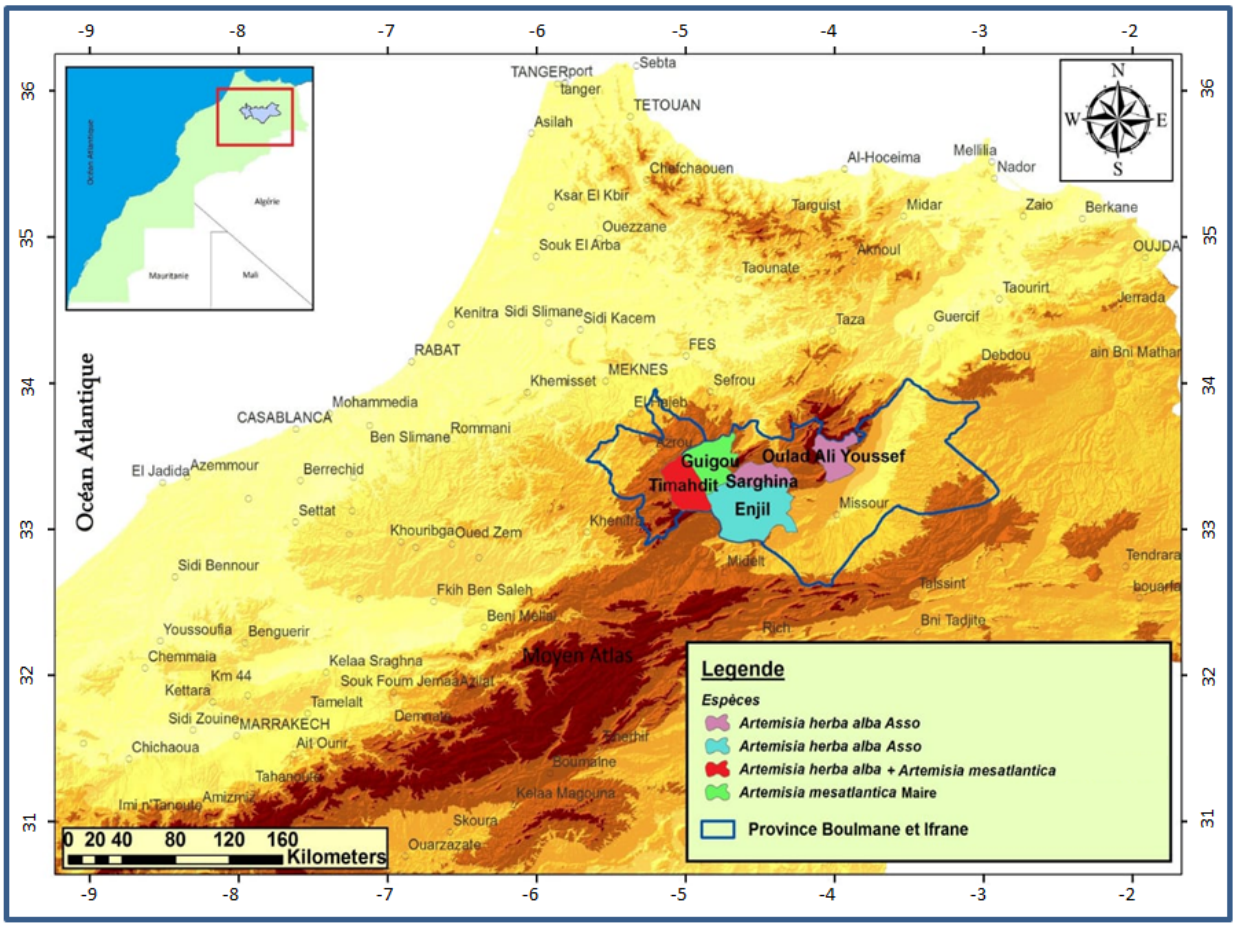

Figure 1. Map showing the harvesting sites of the two sagebrush species.

(**A**) (**B**)

Figure 2. Morphological aspects of *A. herba alba* Asso (**A**) and *A. mesatlantica* Maire (**B**).

2.3. Determination of the Humidity Level

The water contents of the samples collected were determined by the oven-drying process, through measuring the mass of the fresh plant and its mass after drying. Three repetitions were performed.

The humidity rate was calculated by the following relationship:

$$RH\% = \left(\frac{M_0 - M_1}{M_0}\right) \times 100$$

where M_0 is the initial mass of the plant and M_1 is the mass after drying.

2.4. Extraction of Essential Oils and Determination of Yields

The extraction of essential oils (EOs) was carried out by hydrodistillation, from 100 g of flowering tops of each species, for four hours using a Clevenger-type device. Next, the recovered EOs were dried with anhydrous sodium sulfate and then quantified and stored at a temperature of 4 °C, protected from light until their use [8]. This operation was repeated three times.

The EO yield was calculated from 100 g plant material by the formula:

$$\textbf{Yield}(\%) = \frac{V(HE)}{100 - (100 \times TH\%)} \times 10$$

where V (HE) is the volume of HE recovered (mL) and TH (%) is the moisture rate.

2.5. Analysis and Identification of the Chemical Composition of EO

Analysis of the chemical composition of sagebrush EOs was carried out at the Faculty of Sciences research center of Meknes-Morocco.

We used a gas chromatograph of the Thermo Electron type (Trace GC Ultra), coupled to a mass spectrometer of the Thermo Electron Trace MS system type (Thermo Electron: Trace GC Ultra; Polaris Q MS). Fragmentation was carried out by electronic impact with an intensity of 70 eV; the chromatograph was equipped with a DB-5-type column (5% phenyl-methyl-siloxane) (30 m × 0.25 mm × 0.25 µm film thickness) of a flame ionization detector (FID) supplied with a mixture of H2 gas/air. The temperature of the column was programmed at the rate increase of 4 °C/min from 50 to 200 °C for 5 min. The injection mode was split (leakage ratio: 1/70, flow mL/min), and the carrier gas used was nitrogen with a flow rate of 1 mL/min.

Identification of the EO chemical composition was performed through the determination and comparison of compounds' Kovats (IK) indices with those of standard products known from the literature [9–11]. The identification of each compound was made using the Kovats index by comparing peak retention times with those of known authentic standards available in the authors' laboratory, and comparing their reported RI and MS data with those stored in the standard mass spectral databases of Wiley and NIST 14 and the published literature. Kovats indices compare the retention time of any product with the retention time of a linear alkane containing the same carbon number. They are determined by co-injecting a mixture of the alkanes (C7-C40 standard) under the same operating conditions.

2.6. Statistical Analysis

In this study, all statistical analyses were performed by GraphPad Prism 7.03 software (GraphPad Software Inc., San Diego, CA, USA) and XLSTAT v.2017 (Addinsoft, New York, NY, USA), respectively, for statistical comparisons by one-way analysis of variance (ANOVA) and for principal component analysis (PCA) and hierarchical cluster analysis (HCA). Briefly, the ANOVA was performed with multiple Bonferroni and Tukey comparisons tests to compare the extraction yields of essential oils. Any statistical difference was noted if the p-value < 0.5 in Table 1 (*: $p < 0.5$; **: $p < 0.1$; ***: $p < 0.01$). The chemical compositions were subjected to PCA and HCA to determine the degree of correlation of the main compounds identified with particular essential oils (and through this correlation, to deduce the similarity or dissimilarity between species from different localities). HCA was made to group similar compositions of oils using Ward's method and Euclidian distances.

3. Results and Discussion

3.1. Extraction Yields of Essential Oils

The EO yields of each species obtained are presented in Table 1. We note that the essential oil contents of the different species vary from one to another and within the same species, depending on the location of harvest, altitude, soil type and climate; they are included between 0.8% and 2.2% (mL/100 g) (Table 1).

Table 1. Yield (%) in EO of the different species of sagebrush studied.

Species	Codification	Site	EO (Yield %)	Geographical Coordinates and Altitude	Type of Soil	Bioclimatic Atmosphere
Artemisia herba alba Asso	AHAass-OA	Oulad aliyoussef	0.9 ± 0.04 ***	32°24′8.86″ N 3°58′25.98″ W 1507 m	Poor loamy and stony soil	Semi-arid
Artemisia herba alba Asso	AHAass-SA	Serghina	1.0 ± 0.04 ***	33°26′40.955″ N 4°58′8.006″ W 1532 m	Limestone	Semi-arid, arid
Artemisia herba alba Asso	AHAass-ENJ	Enjil	1.1 ± 0.05 ***	33°11′22.068″ N 4°32′0.065″ W 1552 m	Limestone	Semi-arid
Artemisia herba alba Asso	AHAass-TI	Timahdit	1.9 ± 0.09 ***	33°19′36.686″ N 45°51′4.078″ W 1599 m	Limestone	Subhumid to humid
Artemisia mesatlantica Maire	AMma-GU	Guigou	2.2 ± 0.10 ***	33°19′44.637″ N 4°54′31.54″ W 1731 m	Rocky soil on limestone	Subhumid to humid
Artemisia mesatlantica	AM-TI	Timahdit	0.8 ± 0.02 ***	33°24′27.53″ N 4°52′13.49″ W 1498 m	Limestone	Subhumid to humid

***: $p < 0.01$ at least one time by performing comparison between two essential oils yields.

The best EO yield (2.2 ± 0.1%) was given by the AMma-GU sample, followed by AHAass-TI (1.9 ± 0.09%) and finally by AHAass-ENJ (1.1 ± 0.05%). Low levels were recorded for AHAass from the two provinces of Oulad aliyoussef and Serghina, of 1 ± 0.04% and 0.9 ± 0.04%, respectively. Finally, AM-TI provided the lowest EO yield (0.8 ± 0.02%).

From these results, we noted that:

- The value of the sagebrush EO yield increased with the altitude of the sampling site;
- The soil profile was characterized by a dominance of limestone at the levels of AHAass-SA, AHAass-ENJ, AMma-GU, AHAass-TI and AM-TI, while the site of Oulad aliyoussef was of a poor silty and stony type;
- The spontaneous species of sagebrush in humid and subhumid stages exceeded the spontaneous species in arid and semi-arid stages.

A comparison with the literature showed that the yield of AMma-GU (2.2%) from the Boulemane region was higher than that given by Rouani et al. (0.9%) for the same species from the Ifrane region [12]. The EO yields of AHAass from the four provinces Oulad aliyoussef (0.8%), Serghina (0.9%), Timahdit (1.1%) and Enjil (1.9%) were in agreement with those found by Ghanmi et al. (0.8%) [13] for *A. herba alba* of Guercif (Eastern Morocco), by Ourid et al. (1.1%) from the Imouzzer Marmoucha region [14] and close to those obtained by Hinane et al., who showed that *A. herba alba* from Amskroude provided an EO yield of approximately 1.6% [15]. Finally, for AM-TI (Ifrane region), the EO content was higher than that of the Ifrane-Boulmane region (0.5%) [16].

We found that the EO yields obtained for other sagebrush species from western and southwestern Turkey, such as *A. vulgaris* L. (0.4%), *A. santonicum* L. (0.4%) and *A. campestris* (0.7%) [17], remained significantly lower than those of the sagebrush species in the present study.

From these results, it can be concluded that intra-specific variations in yields appear to be correlated with abiotic factors such as the climate specific to the sample collection region and geographic factors such as the elevation and soil type. This agrees with the results reported by El Idrissi et al. (2016), according to which variations in content can be caused by several factors including the climate, altitude and soil type. Several studies have also shown the influence of the vegetative cycle, nature of the plant (dried or fresh), period of picking and extraction method on the yield and quality of the essential oil [18–20].

3.2. Chemical Composition of the Studied Sagebrush Essential Oils

Tables 2 and 3 and the histogram in Figure 3 present the results related to the chemical compositions of the essential oils of the six *Artemisia*, determined by GC/SM.

Table 2. The chemical composition of essential oil of *A. herba alba* Asso from different locations.

Component	KI[a]	KI[b]	Limestone/ Semi-Arid	Poor Loamy and Stony Soil/ Semi-Arid	Limestone/ Semi-Arid, Arid	Limestone/ Subhumid to Humid
Santolinatriene	908	909	1.1	–	–	–
Tricyclene	926	925	0.2	–	–	–
α-Thujene	930	924	–	0.9	–	–
α-Pinene	939	937	0.1	–	–	–
Camphene	954	952	1.6	2.5	–	–
Sabinene	975	974	–	4.3	–	–
β-Pinene	979	979	0.5	–	–	–
1-Decene	989	988	–	–	0.7	–
Yomogi alcohol	999	999	3.8	–	–	–
α-Terpinene	1017	1014	–	2.3	–	–
ρ-Cymene	1024	1023	–	5.9	–	–
Allyltiglate	1025	1022	–	–	0.43	–
o-Cymene	1026	1027	0.9	–	–	0.6
1,8-Cineole	1031	1024	13.8	1.0	–	1.4
Santolina alcohol	1040	1038	7.3	–	–	–
Bergamal	1056	1056	0.2	–	–	–
γ-Terpinene	1059	1060	–	4.1	–	0.5
cis-Sabinene hydrate	1070	1067	–	0.9	–	–
Artemisia alcohol	1083	1083	1.4	–	–	–
Terpinolene	1088	1089	–	0.8	–	–
cis-Thujone	1102	1102	3.4	–	–	5.5
trans-Thujone	1114	1110	–	8.9	0.4	33.8
neo-Isopulegol	1120	1142	–	1.5	–	–
Chrysanthenone	1127	1126	–	2.8	–	–
trans-*p*-Menth-2-en-1-al	1136	1136	0.8	–	–	–
trans-Pinocarveol	1139	1141	0.4	–	–	1.0
trans-ρ-Menth-2-en-1-ol	1140	1120	–	1.1	–	–
trans-Verbenol	1144	1145	–	0.9	–	–
Camphor	1146	1143	46.2	18.4	0.8	2.0
Citronellal	1153	1159	0.3	–	–	–
iso-Isopulegol	1159	1149	–	1.6	–	–
Pinocarvone	1164	1164	1.2	–	–	–
Chrysanthenol	1164	1163	4.8	–	–	–
Borneol	1169	1169	2.9	3.5	–	–
4-hexen-1-ol,5-methyl-2-(1-methylethenyl) Lavandulol	1172	1171		0.2	–	–
Artemisyl acetate	1173	1173	–	–	1.2	–
Terpinen-4-ol	1177	1177	–	8.6	–	1.7
Thuj-3-en-10-al	1184	1181	1.3	1.2	–	–
α-Terpineol	1188	1189	1.0	–	–	1.3
Myrtenol	1195	1195	0.6	1.6	–	–
cis-Piperitol	1196	1198	0.3	–	–	–
γ-Terpineol	1199	1199	0.3	–	–	–
trans-Piperitol	1208	1198	0.4	–	–	–
neoiso-Dihydrocarveol	1228	1220	–	–	–	0.6
Cumin aldehyde	1241	1242	–	1.1	–	–
trans-Piperitone epoxide	1256	1254	0.4	–	–	–
cis-Chrysanthenylacetate	1265	1266	–	3.3	0.6	–
Isobornylacetate	1285	1290	0.8	–	–	–

Table 2. *Cont.*

Component	KI[a]	KI[b]	Limestone/ Semi-Arid	Poor Loamy and Stony Soil/ Semi-Arid	Limestone/ Semi-Arid, Arid	Limestone/ Subhumid to Humid
Lavandulylacetate	1290	1291	0.2	1.0	–	1.8
Myrtenylacetate	1326	1325	2.1	–	–	–
ρ-Mentha-1,4-dien-7-ol	1327	1332	–	2.5	–	–
Piperitenone	1343	1343	–	2.3	–	–
α-Terpinylacetate	1349	1349	–	–	–	0.4
Eugenol	1359	1356	–	2.6	0.4	–
cis-threo-Davanafuran	1415	1414	–	–	1.7	–
(*E*)-Caryophyllene	1419	1433	–	–	–	0.8
Dictamnol	1429	1428	–	–	–	3.5
Vestitenone	1446	1443	–	–	4.9	–
exo-Arbozol	1454	1452	0.3	–	–	–
dehydro-Sesquicineole	1471	1460	–	–	–	0.6
Germacrene D	1481	1488	–	–	0.5	0.8
Davana ether	1491	1491	–	3.0	14.7	–
β-Himachalene	1500	1499	–	–		0.4
Laciniatafuranone F	1532	1503	–	–	1.6	–
Artedouglasiaoxide A	1535	1533	–	–	1.5	–
Furopelargone A	1540	1540	–	–	3.3	–
Laciniatafuranone E	1542	1514	–	–	9.8	–
Laciniatafuranone H	1550	1532	–	–	0.4	–
Artedouglasia oxide D	1560	1560	–	–	0.5	–
Davanone B	1566	1565	–	2.0	10.1	–
α-Cedreneepoxide	1575	1617	–	1.0	–	–
Spathulenol	1578	1619	0.9	1.6	6.2	–
Artedouglasia oxide B	1582	1580	–	–	0.6	–
Caryophyllene oxide	1583	1582	–	1.2	1.0	1.3
Davanone	1587	1586	–	3.6	13.6	–
Globulol	1590	1590	0.3	–	–	1.8
Viridiflorol	1592	1595	–	–	0.3	–
Rosifoliol	1600	1599	–	–	–	2.4
Humulene epoxide II	1608	1608	–	–	0.5	–
Davanol D1 (isomer 1)	1615	1615	–	–	3.4	–
Junenol	1619	1605	–	–	–	1.2
trans-Isolongifolanone	1626	1627	–	–	–	0.7
α-epi-Cadinol	1640	1640	–	–	–	0.7
α- epi-Muurolol	1642	1642	–	–	–	6.4
α-Bisabololoxide B	1658	1656	–	–	–	7.4
α-Bisabolol	1685	1685	–	–	–	0.8
α-Germacra-4(15),5,10(14)-trien-1-ol	1686	1686	–	–	0.3	–
Deodarone	1698	1707	–	–	–	5.2
β-(Z)-Santalol	1716	1713	–	–	–	0.6
α-Bisabololoxide A	1749	1686	–	–	–	6.8
Eremophilone<8-hydroxy-dihydro->	1757	1774	–	–	4.3	–
Vetivenic acid	1811	1860	–	–	14.9	7.8
Eremophilone<8-hydroxy->	1847	1865	–	2.0	–	–
Carissone	1927	1926	–	–	–	0.5
Identification total (%)			100	100	99.9	100
Oxygenated monoterpenes (%)			94.9	64.7	11.4	49.4
Sesquiterpene hydrocarbons (%)			0	0	0.5	1.9
Oxygenated sesquiterpenes (%)			1.2	14.5	87.4	47.6
Monoterpene hydrocarbons (%)			4.4	20.8	0.7	1.1

KI: Kovats retention index; KI[a]: Determined; KI[b]: Reported index from literature (Adams, 2017, NIST Chemistry Web Book and Wiley libraries).

Table 3. The chemical composition of essential oil of *A. mesatlantica* from different locations.

Component	KI[a]	KI[b]	Rocky Soil on Limestone/ Subhumid to Humid	Limestone/ Subhumid to Humid
Santolinatriene	908	909	1.0	–
Tricyclene	926	925	0.2	–
α-Thujene	930	924	0.3	–
α-Pinene	939	937	–	0.4
Camphene	954	952	4.5	0.3
Sabinene	975	974	–	4.5
B-Pinene	979	979	0.4	0.2
1-Decene	989	988	–	–
Myrcene	990	991	–	0.2
Yomogi alcohol	999	999	3.5	–
α-Terpinene	1017	1014	–	0.1
ρ-Cymene	1024	1023	–	0.7
o-Cymene	1026	1027	1.1	–
1,8-Cineole	1031	1024	13.2	3.7
Santolina alcohol	1040	1038	5.9	–
β -(E)-Ocimene	1050	1050	0.3	–
Bergamal	1056	1056	0.4	–
Artemisia ketone	1062	1061	1.1	–
cis-Sabinene hydrate	1070	1067	–	1.5
cis-Thujone	1102	1102	–	4.7
trans-Thujone	1114	1110	3.1	41.0
neo-Isopulegol	1120	1142	0.5	–
Chrysanthenone	1127	1126	1.1	–
allo-Ocimene	1132	1131	–	0.2
trans-Pinocarveol	1139	1141	0.4	–
trans-ρ-Menth-2-en-1-ol	1140	1120	0.4	–
trans-Sabinol	1142	1140	–	3.6
Camphor	1146	1146	44.9	3.2
Pinocarvone	1164	1164	1.1	0.2
Borneol	1169	1169	2.9	1.0
4-hexen-1-ol,5-methyl-2-(1-methylethenyl) Lavandulol	1172	1171	0.2	–
Terpinen-4-ol	1177	1177	1.1	1.6
Thuj-3-en-10-al	1184	1181	–	0.2
Prenylangelate	1190		0.3	–
Myrtenal	1194	1193	0.6	–
Myrtenol	1195	1195	0.3	0.3
γ-Terpineol	1199	1199	0.3	1.0
trans-Piperitol	1208	1198	0.4	0.4
endo-Fenchyl acetate	1220	1220	–	0.8
Piperitone	1252	1253	0.3	0.2
cis-Chrysanthenylacetate	1265	1266	2.2	–
Isobornylacetate	1285	1290	0.8	–
exo-Arbozol	1454	1452	0.3	–
Sesquicineole<7-epi-1,2-dehydro->	1473	1460	–	1.3
Germacrene D	1481	1488	–	2.7
Spathulenol	1578	1619	1.1	1.6
Globulol	1590	1590	0.3	1.6
Viridiflorol	1592	1595	0.4	
Eremoligenol	1631	1630	–	6.7
α-Cadinol	1654	1653	–	3.2

Table 3. *Cont.*

Component	KI[a]	KI[b]	Rocky Soil on Limestone/ Subhumid to Humid	Limestone/ Subhumid to Humid
Botrydiol	1690	1689	–	3.3
Identification total (%)			99.7	99.9
Oxygenated monoterpenes (%)			90.4	63.6
Sesquiterpene hydrocarbons (%)			0	2.7
Oxygenated sesquiterpenes (%)			1.8	26.8
Monoterpene hydrocarbons (%)			7.5	6.9

KI: Kovats retention index; KI[a]: Determined; KI[b]: Reported index from literature (Adams, 2017, NIST Chemistry Web Book and Wiley libraries); % = Percentage composition of each compound; (–): Not identified.

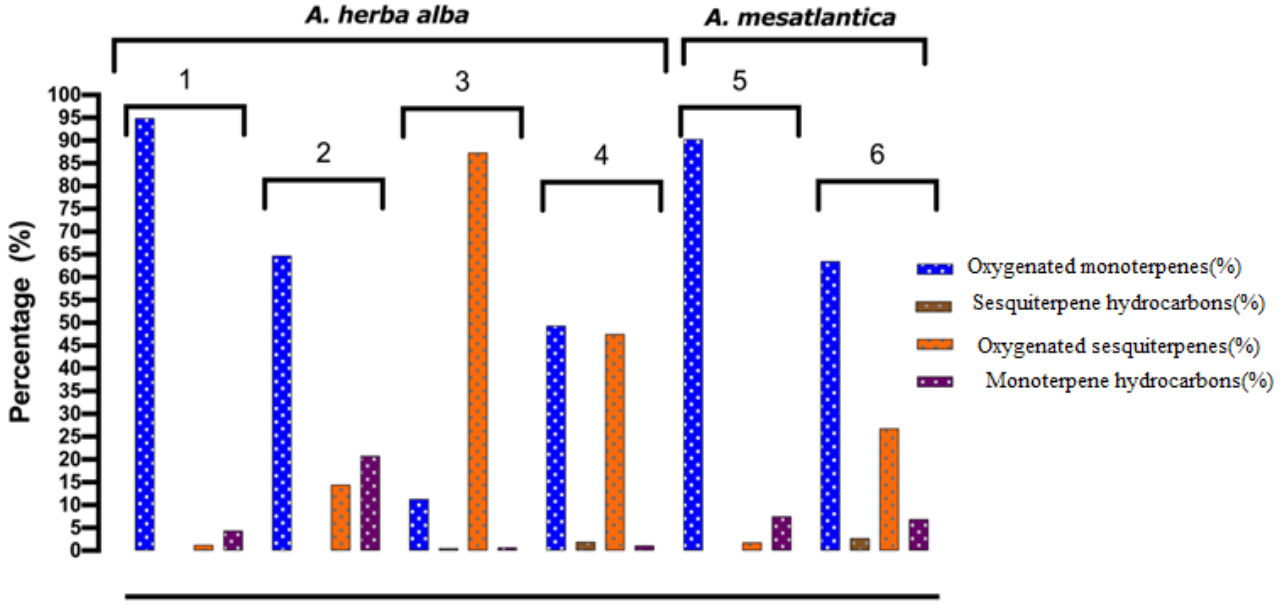

Figure 3. Different classes of compounds identified in the EOs of *A. herba alba* Asso: (**1**) Limestone/Semi-arid; (**2**) Poor loamy and stony soil/Semi-arid; (**3**) Limestone/Semi-arid, arid; (**4**) Limestone/Subhumid to humid, *A. mesatlantica*; (**5**) Rocky soil on limestone/Subhumid to humid; (**6**) Limestone/Subhumid to humid.

Analysis of the results of the chemical compositions of essential oils of sagebrush from the Boulemane and Ifrane regions revealed the presence of 34 chemical compounds for the two species *A. mesatlantica* Maire (Guigou) and *A. herba alba* Asso (Enjil), 33 for *A. herba alba* Asso (Oulad aliyoussef) and *A. mesatlantica* (Timahdit), 31 for *A. herba alba* Asso (Timahdit) and 28 for *A. herba alba* Asso (Serghina), whose total chemical composition varies between 99.7% and 100% of the total EO (Tables 3 and 4). These compounds divide into four main chemical families:

- Oxygenated monoterpenes (11.4 to 94.9%), of which the major compound is camphor (0.82 to 46.19%) followed by *trans*-thujone (0.4 to 46.2%). Then come 1,8-cineole (0.9 to 13.8%), and in a small percentage, *cis*-thujone (3.5 to 5.5%);
- Hydrocarbon monoterpenes (0.7 to 20.8%), which are mainly represented by camphene as the major compound (0.3 to 4.5%);
- Hydrocarbon sesquiterpenes (0.51 to 2.74%), the most important of which are present in AM-TI *Artemisia* with a percentage of 2.74%. However, we note the absence of the latter in the species AHAass-NJ, AMma-GU and AHAass-OA;
- Oxygenated sesquiterpenes, which reach (87.4%) of the EO of AHAass-SA, represented by vetivenic acid (14.9%), davana ether (14.6%) and davanone (13.1%). We were

the first to identify the two compounds vetivenic acid and davana ether within the sagebrush species. The latter is also present in the EOs of AHAvar-TI (47.6%), AHAass-OA (14.5%) and AM-TI (26.8%), and with lower percentages, in AHAass-NJ (1.2%) and AMma-GU (1.8%).

Table 4. Overall percentages (%) of the different chemical families identified in the EO of *A. herba alba* Asso: Oulad aliyoussef, Serghina, Enjil and Timahdit; *A. mesatlantica* Maire: Guigou and Timahdit.

Components	*A. herba alba* Serghina	*A. herba alba* Oulad Ali Youssef	*A. herba alba* Enjil	*A. herba alba* Timahdit	*A. mesatlantica* Timahdit	*A. mesatlantica* Guigou
Alcohols (%)	6.9	26.6	24.5	20.8	32.8	22.7
Aldehydes (%)	0	2.3	3.2	0	0.1	1.0
Ketones (%)	31.3	39.9	50.7	55.4	49.5	51.7
Esters (%)	16.4	6.3	2.9	0.4	2.9	0.8
Oxides (%)	4.1	5.1	13.8	12.9	13.2	5.7
Phenols (%)	0	0	0	1.76	0	0

We noted the presence of certain oxygenated monoterpenes in the EO of AHAass-NJ but at lower contents: santolina alcohol (7.3%), chrysanthenol (4.6%), yomogi alcohol (3.8%) and borneol (2.8%).

Other monoterpene hydrocarbons were also identified, such as *o*-cymene (5.9%) and sabinene (4.3%), in the EO of AHAass-OA. It is important to note that the percentages of the chemical compounds of AHAass-NJ and AMma-GU are similar to the other studied species collected in the same region.

Examination of Tables 2–4 showed that:

A. herba alba has four different chemotypes: *trans*-thujone and *iso*-acorone extracted from the plant from Timahdit and camphor and 1,8-cineole from Enjil. However, *A. herba alba* Asso combines camphor and *trans*-thujone from Oulad aliyoussef, and conversely, Serghina is predominated by vetivenic acid and davana ether.

In *A. mesatlantica* of Guigou, camphor and 1,8-cineole predominate, while *A. mesatlantica* from Timahdit is *trans*-thujone and occidenol.

Overall, the constituents of our isolated oils are complex mixtures of monoterpene hydrocarbons, alcohols, aldehydes, ketones, oxides, esters and a very low rate of phenols, which are characterized by the predominance of terpene products with ketone structures (camphor and thujone, in particular).

The results obtained show remarkable variations in the chemical composition of different species, within the same genus, within the same species and between provinces. These differences are remarkable, in particular, due to the nature of the most abundant compound and the other constituents identified; even if we find some similar elements, this variability from one locality to another confirms that the chemical profile of the species studied is very variable depending on the origin of the plant, the altitude, the nature of the soil and the climate specific to the region. Other factors influence the chemical composition, affected by factors such as the season, environmental conditions [1] and genetic makeup [6].

3.3. Analysis of the Similarity between Species by Ascending Hierarchical Analysis (HCA) and Principal Components (PCA)

Analysis of the chemical composition of the essential oils of the sagebrush studied (*A. mesatlantica* Maire, *A. herba alba* Asso) showed a high diversity of the compounds identified, both qualitatively and quantitatively. For statistical description of our sampling and to be able to highlight a possible chemical variability, as well as to identify the possible relationships between the abundance of volatile organic compounds and the abiotic and geographical factors, we used two common tests of multidimensional descriptive statistics: bottom-up hierarchical analysis and principal component analysis (PCA).

3.4. Hierarchical Bottom-Up Analysis

First, a bottom-up hierarchical analysis was performed to divide the sample into groups of homogeneous observations, each group being well-differentiated from the others. This analysis then brought the samples together to produce a dendrogram or classification tree (Figure 4).

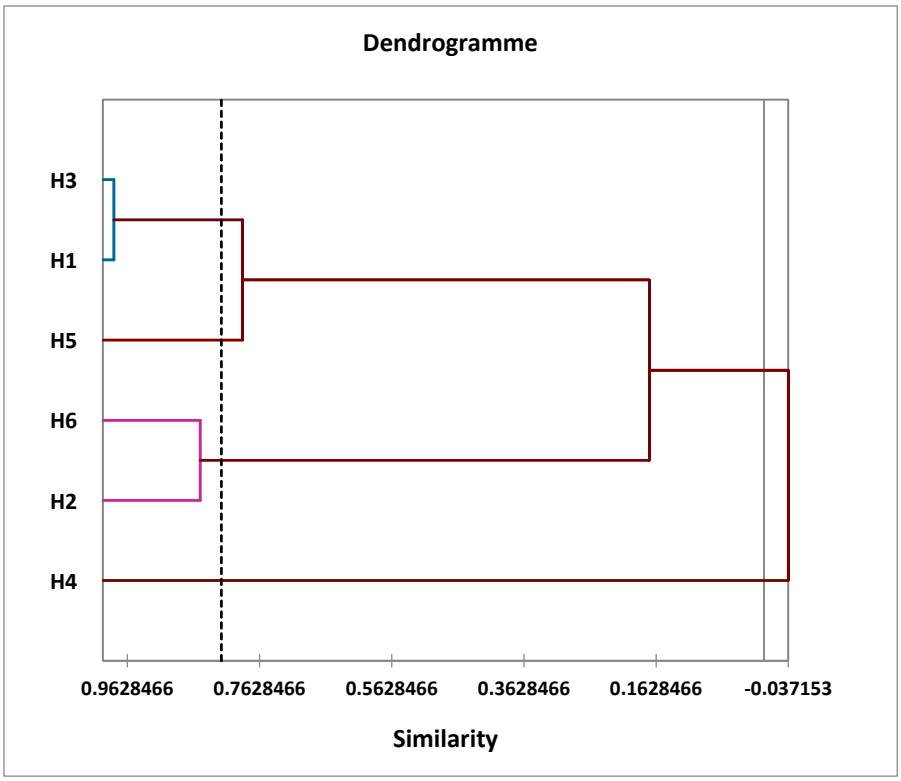

Figure 4. Dendrogram obtained by a hierarchical bottom-up analysis (HCA) of the six samples studied.

Figure 4 shows the similarity between the EOs of the six samples studied. This analysis shows that the samples can be grouped into four distinct groups depending on the distance between them:

- The first group (I) includes two oil samples: H1 and H3;
- The second group (II) also contains two samples: H2 and H6;
- The third group (III) comprises a single sample: H4;
- The fourth group (IV) also contains a single sample: H5.

At the dendrogram level, we noted that the EOs of group (I) included two samples of oils and those were grouped within the same class, so there was intra-class homogeneity; this was confirmed by the small distance between the two populations, which explained why the two taxa were similar or very close in terms of the chemical profile example:

- H3 has 98.31% similarity with H1;

Likewise, Group (II) included two samples of oils that were similar or very similar in their chemical profiles:

- H6 has 85.25% similarity with H2;

In contrast, for group (III) and (IV), these consisted of a single oil, H4 or H5. They were heterogeneous compared to other classes, with a significant distance.

- H6 has 17.32% similarity with H1 and H3.

Note:

H1: *A. herba alba from the Enjil region;*

H2: *A. herba alba from the Timahdit region;*

H3: *A. mesatlantica from the Guigou region;*

H4: *A. herba alba from the Serghina region;*

H5: *A. herba alba from the Oulad Ali region;*

H6: *A. mesatlantica from the Timahdit region.*

3.5. Principal Component Analysis (PCA)

Principal component analysis (PCA) is a method that allows for the simplification of data by studying the relationships between all variables, to determine the similarities and dissimilarities between individuals (Figures 5 and 6).

To perform this analysis, we chose the first two factor axes. The dispersion of the *Artemisia* L. species in the plane formed by these two axes concerning the chosen variables explained 77.62% of the variability, including 48.13% on the first axis and 29.49% on the second one (Figure 6). This figure confirmed the results of the bottom-up hierarchical analysis and highlighted the main quantitative differences between the four chemical composition groups. Furthermore, it showed that the chemical composition of essential oils from different populations is heterogeneous.

On the other hand, some chemical compounds were positively correlated, for example, essential oils (H6, H2) that were rich in *trans*-thujone contained less camphor, iso-acorone, α-bisabolol oxide β and α-bisabolol oxide A. Thus, as essential oils (H1, H3) that are rich in camphor, 1,8 cineoles are also rich in davanone, *cis*-thujone and camphene. We noted that certain species had similar compositions, such as H2 and H6. On the other hand, others presented a significant difference in terms of chemical composition, such as H4 and H5. In addition, we noted that there were several correlations between chemical compounds with certain EOs. We can cite compound 16 (camphor), which strongly correlated with EOs H1 and H3 and very weakly correlated with EOs H2 and H6. Regarding compound 11 (*trans*-thujone), contrary to compound 16 (Camphor), it relatively highly correlated with EOs H6 and H2 and very weakly correlated with EOs H1 and H3.

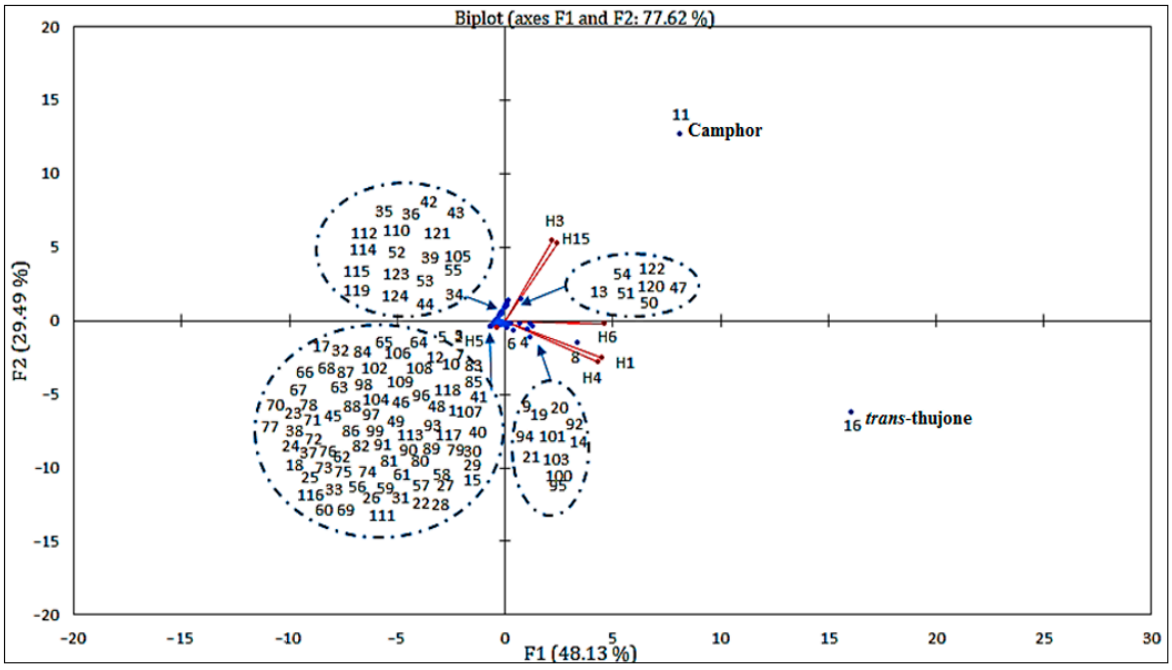

Figure 5. Correlation between the principal compounds and the essential oils of the six samples from different regions studied.

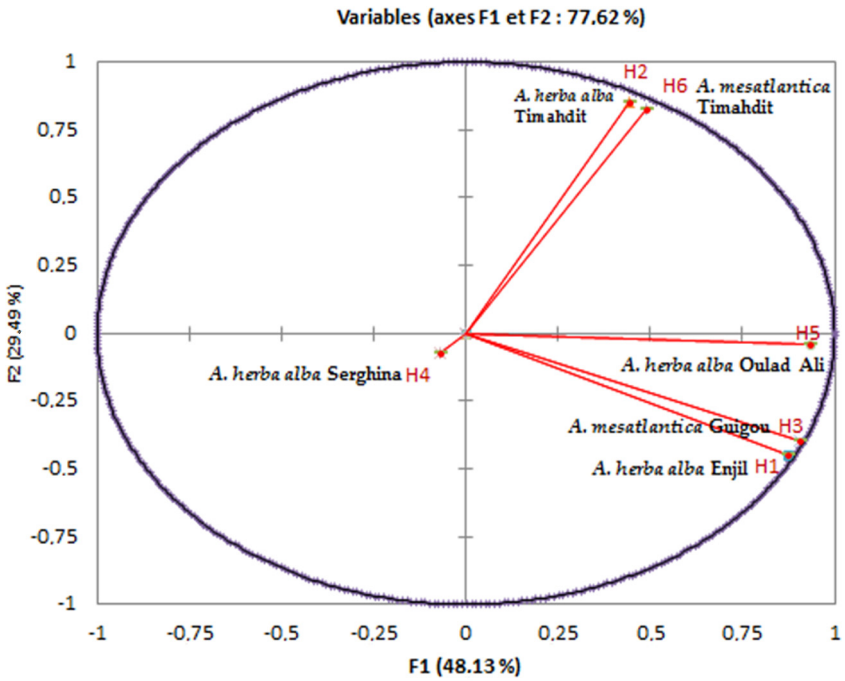

Figure 6. Principal component analysis for the EO of the six samples from the different regions studied.

We deduced that the chemical compositions of the six samples of essential oils collected in different regions of Morocco presented heterogeneity; indeed, some samples contained camphor as the major compound, while others contain *trans*-thujone. The contents of other compounds 1,8-cineole, vetivenic acid, davana ether, occidenol and *iso*-acorone varied substantially. Although the compositions of these samples were largely dominated by camphor and *trans*-thujone, statistical analysis distinguished four groups:

- The first group (I) was rich in camphor (compound 11) and 1,8-cineole (compound 8); this group contained two samples, one from Enjil: AHAass-ENJ (1552 m), and another from Guigou: AMma-GU (1731 m);
- The second group (II) contained two samples from the same region (Timahdit), AHAass-TI (1599 m) and AM-TI (1498 m), characterized by an abundance of trans-thujone (compound 16);
- The third group (III) comprised a single sample from Serghina: AHAass-SA (1532 m), characterized by particular abundances of vetivenic acid and davana ether;
- The fourth group (IV) also contained a single sample from Oulad aliyoussef, AHAass-OA (1507 m), characterized by particular abundances of camphor and *trans*-thujone.

Previous studies found similar chemical compositions for the sagebrush essential oils in our study, where camphor and *trans*-thujone were the primary compounds.

The presence of camphor as a major constituent in the EOs of *A. herba alba* Asso d'Oulad Ali, *A. herba alba* from Enjil and *A. mesatlantica* Maire de Guigou has already been mentioned in certain populations of *A. herba alba* in Morocco [21,22], Spain [23] and Algeria [24] and for other *Artemisia* species worldwide: *A. absinthium*. L in Brazil [25], *A. sieberi* in Iran [26] and *A. nilagirica* in India [27].

Similarly, for *A. mesatlantica* (Timahdit) and *A. herba alba* Asso (Timahdit), the chemical compositions were found to be similar to those in other work carried out in Morocco on *A. mesatlantica* [12,28], in India on *A. nilagirica* [29] and in Tunisia on *A. herba alba* [30], where trans-thujone is the major constituent. However, in other works, the results were quite different. EOs of *A. anethoides* were dominated by 1,8-cineole [31] in China, *A. judaica* was the predominant piperitone in Jordan [32] and α-thujone was most prevalent in the EO of *A. herba alba* in Tunisia [33]. Meanwhile, davanone has been described as an important component in EO of *A. herba alba* from Morocco [34].

4. Conclusions

Essential oils are substances with a very complex chemical composition. Within the same genus *Artemisia*, our study has shown qualitative and quantitative variations in the yields and chemical compositions of the essential oils studied.

In general, the observed differences clearly show the influence of abiotic factors such as the climate specific to the regions where samples were collected and geographical factors such as the altitude and nature of the soil.

We also noted in this study a slight modification of the chemical compositions of the studied species. This modification was remarkable at the level of the major compounds, i.e., *trans*-thujone (41.1%), camphor (46.2%) vetivenic acid (14.9%) or 1,8-cineole (13.8%). HCA affirmed the findings of PCA and allowed us to distinguish four groups. Thus, at the end of this study, it appears that the *Artemisia* species are rich in terpene products of ketone structures (camphor and thujone, in particular), which are bioactive molecules responsible for various biological activities and highly sought after in the pharmaceutical, cosmetic and industrial fields. The promotion of these plants in the aforementioned sectors can contribute to the promotion and social and sustainable development of the medicinal and aromatic plants (MAP) sector. In fact, the MAP sector in this locality needs promotion to support its marketing, processing techniques, labeling and certification. Such promotion would be of great interest to the regions studied and could even be a source of significant income. This would allow, among other things, the local population to market their local products and develop them to be healthier. Yet, such progress can only be made possible by ethnopharmacological, phytochemical and biological studies determining the scientific names of the *Artemisia* species, the yields of EOs, their qualitative and quantitative chemical compositions and the biological properties of the chemical compounds of sagebrush species in the regions studied.

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